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16. ABSTRACT

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ABSTRACT

Two second-order transformation rate coefficients -- k_b, based on total plate counts, and k_A, based on periphyton-colonized surface areas -- were used to compare xenobiotic chemical transformation by laboratory-developed (microcosm) and by field-collected microbiota. Similarity of transformer to non-transformer community structure in blended aufwuchs was indicated by k_b values, and similarity of transformation rates per unit of periphyton-colonized surface area was indicated by k_A values. Xenobiotic chemicals used for the comparisons were methyl parathion (MP), diethyl phthalate (DEP), and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE). Even though rate coefficients of each of the chemicals were similar among microcosm- and field-collected microbial samples, not all of the field-collected samples showed MP or DEP transformation. MP transformation was suppressed by aqueous extracts of field-collected, algae-dominated aufwuchs and by low concentrations of DEP. Lack of DEP transformation appeared to have resulted from an absence of DEP-transforming bacteria in field-collected aufwuchs samples.

KEY WORDS: microcosm, microbial degradation, microbial transformation, methyl parathion, diethyl phthalate, 2,4-DBE, mathematical model.

INTRODUCTION

Laboratory microcosms are used routinely to simulate environmental waters for research on the transport, fate, and effects of pollutants. We have used aquatic microcosms in the form of large, flowing laboratory ecosystems for developing and testing rate coefficients for microbial transformations of xenobiotic chemicals (1, 2). Our laboratory ecosystems were not intended to simulate particular rivers, lakes or streams but were designed to provide controlled aquatic systems having a wide diversity of physical,

chemical, and biological parameters. The diverse laboratory environments were used to test the applicability of rate coefficients under a wide range of conditions. Although our laboratory ecosystems were not designed to simulate any particular field sites, it is essential that they provide ranges in physical, chemical, and biological characteristics similar to those found in the natural environment. Such a comparability in ranges of characteristics between field and laboratory systems is required if the rate coefficients developed and tested with laboratory systems are to have applicability to diverse field situations.

In developing and testing microbial transformation rate coefficients, we wanted to be sure that our laboratory ecosystem microbiota were similar to microbial populations found in natural waters. To test the comparability, we used two second-order transformation rate coefficients: k_b, based on total plate counts of bacteria, and k_A, based on a ratio of colonized surface area to container volume. Because our laboratory ecosystems were dominated by aufwuchs (microbiota attached to surfaces and floating in mats), we compared transformation rate coefficients for field-collected aufwuchs and for laboratory ecosystem aufwuchs. Three xenobiotic chemicals were used in this study: methyl parathion (MP), diethyl phthalate (DEP), and 2,4-dichlorophenoxyacetic acid butoxyethyl ester (2,4-DBE).

Blended aufwuchs was used for k_b determinations to provide a basis for testing the diversity of bacterial populations. We had previously shown that proportions of transformer to non-transformer bacteria varied spatially and temporally in aufwuchs samples, causing several orders of magnitude of variation in k_b for blended aufwuchs samples (1, 2). Therefore, k_b provided a basis for determining whether laboratory ecosystems and field aufwuchs had similar ranges in ratios of transformer to non-transformer bacteria.

A second rate coefficient, k_A, based on the ratio of colonized surface area to container volume, was shown to be relatively constant under diverse laboratory ecosystem conditions (2). Therefore, k_A provided a basis for determining whether laboratory ecosystem periphyton had similar microbial transformation rates per unit of colonized surface area compared to field periphyton. In summary, we used two second-order transformation rate coefficients to test both the diversity of transformer to non-transformer community structure and the similarity of transformation rates per unit of surface area between laboratory and field microbiota.

PROCEDURE

Our experimental design was such that transformation rates were kinetically comparable. The approach was based on Michaelis-Menten kinetics under specitic conditions of no growth and low xenobiotic chemical concentrations, whereby the transformation rates were pseudo-first-order relative to the chemical concentrations. First-order rate coefficients (k_1) were calculated for loss of parent compounds in batch cultures and divided by organism concentrations or colonized surface area to container volume ratios to give second-order rate coefficients k_b and k_A, respectively.

To ensure that transformation rate coefficients were based on data of high precision, k_1 values were used only if their standard error of regression did not exceed 16%. Total plate counts were not used if the coefficient of variation among replicate plates exceeded 30%.

We considered numerous factors pertaining to experimental design for comparing transformation rate coefficients. The most important considerations included (i) xenobiotic chemical concentration, (ii) organism growth, (iii) abiotic reactions, (iv) sorption, (v) classes of organisms performing

the transformation, (vi) phase of organisms responsible for the transformation, (vii) adaptation, (viii) mass-transport effects, (ix) and colonization times of attached communities. Our approach to each of these considerations was as follows.

Xenobiotic Chemical Concentrations. According to the Michaelis-Menten equation for enzyme-substrate reactions (3), transformation rates for a constant enzyme concentration are first-order relative to substrate concentrations until enzyme saturation is approached. As enzyme saturation is approached, the reaction becomes mixed order, and after complete saturation the reaction is zero order (Fig. 1). So that first-order rate coefficients could be determined, our experiments were conducted at xenobiotic chemical concentrations well below enzyme saturation. These conditions were ascertained by determining that the rates of chemical loss were first-order over approximately two orders of magnitude in concentration, i.e. plots of the logarithms of chemical concentration versus time were linear (Fig. 2).

Organism Growth. To ensure no significant organism growth during the experiments, 10^7 to 10^9 CFU of bacteria per liter were used, and transformation rates were measured during short incubation periods (usually less than 10 h).

Abiotic Reactions. Control batch cultures that had been autoclaved and were at the same pH as test cultures were used to show that abiotic reactions were insignificant compared to biolysis rates under the conditions of the experiments. Microcosms fluctuated in pH during diurnal cycles, reaching a maximum of approximately pH 8; however, chemical hydrolysis rates were insignificant compared to biolysis rates. When necessary, batch cultures were adjusted to approximately pH 7, using HCl or KOH, before starting the experiments.

Sorption. Autoclaved batch cultures containing several grams of biomass (ash-free dry weight, AFDW) per liter were used to show that sorption of the chemicals to biomass was not a significant process.

Identification of Transformers. In our study, bacteria were shown to be the only significant transformers of MP and DEP. 2,4-DBE was rapidly transformed by bacteria, fungi, and algae in our test systems. These conclusions were based on the use of candicidin treatments of mixed populations and by testing axenic cultures of bacteria, fungi, and algae for transformation of 2,4-DBE (1, 2).

Microbial Phase Associations. Sediments were not used in these studies, therefore, microbiota were distributed in only three phases: suspended free-living, suspended attached (to suspended particulates), and attached (to container walls). Based on the total number of bacteria attached to microcosm walls compared to the number of bacteria associated with the water, the microcosms were dominated by the attached phase.

Adaptation. No lag in transformation rates of the chemicals occurred, i.e. logarithmic plots of chemical concentration versus time were linear from the initial time of spiking (Fig. 2). Therefore, no adaptation by induction of enzyme systems was evident during the measurement of transformation rates. Also, no measurable increase in transformation rates occurred in microcosms exposed to the chemicals for several months. Therefore, no adaptation by changes in community structure was evident during the course of the studies.

Mass-Transport Effects. Transformation rates were not mass-transport limited for suspended populations in batch cultures or microcosm systems. This conclusion was based on the observation that transformation rates did not increase for blended aufwuchs samples as turbulence increased by an order

of magnitude. Mass-transport limitation was apparent for attached communities, however, transformation rate coefficients (k_1) were directly proportional to turbulence measured in Reynolds numbers for periphyton attached to Teflon strips (Fig. 3). Therefore, similar turbulences were used for comparison of transformation rate coefficients for attached communities.

Periphyton Colonization Time. Transformation rate coefficients (k_1) for Teflon strips colonized with periphyton increased as colonization time increased (Fig. 3, 4). Colonization rates were much slower in cold water (approximately 5°C) than in warm water (20°C). Strips used in these experiments were colonized at 15 to 25°C and were maturely colonized (as shown by no increase in k_1 for several additional weeks of colonization) after approximately 6 weeks.

With the above experimental design considerations accounted for, several transformation rate coefficients were calculated from batch culture experiments. The rate coefficients included (i) a first-order rate coefficient for loss of the xenobiotic chemical from water over time (k_1), (ii) a second-order rate coefficient based on total bacterial plate counts (k_b), and (iii) a second-order rate coefficient based on a ratio of colonized surface area to container volume (k_A). The rate coefficients were determined using the following procedures.

First-order transformation rate coefficients (k_1) were calculated using published methods (1,2). For the study, batch cultures of blended aufwuchs or intact periphyton attached to Teflon strips were spiked to an initial concentration of approximately 150 µg liter^{-1} MP, 150 µg liter^{-1} 2,4-DBE, or 350 µg liter^{-1} DEP. Concentrations of the chemicals were determined over time by extracting culture samples with 2,2,4-trimethyl pentane and analyzing the extracts by electron capture detection using gas-liquid chromatography. Blended aufwuchs cultures were shaken in an incubator-shaker at 20°C and

intact periphyton cultures were stirred at ambient room temperature by stainless steel paddlewheels. Values of k_1 for continuous-flow microcosms were calculated from xenobiotic steady-state concentrations versus input concentrations. Second-order transformation rate coefficients (k_b) for blended aufwuchs cultures were calculated by dividing k_1 by total plate counts (1,2). Second-order rate coefficients (k_A) for intact periphyton cultures were calculated by dividing k_1 by the ratio of colonized Teflon surface area to container volume (2).

Collection of Microbiota

Field-collected and microcosm microbiota were obtained by colonizing 11 X 15 cm Teflon strips for approximately 10 weeks at the field sites and by collecting aufwuchs grab samples from large laboratory microcosms and field sites. Experiments were started within 24 h after transporting the samples to the laboratory.

Laboratory Microcosms

The laboratory microcosms consisted of two independent flowing channels (designated A and B), each constituting a series of eight 200-liter, continuously stirred tank reactors (CSTRs), designated 1 through 8. The water temperature was 20°C for MP and DEP and 15°C for 2,4-DBE. The channels were lighted with 2000 footcandles (21.5klx) of fluorescent light on 12-h light-dark cycles for MP and DEP, and were lighted with 750 footcandles (8.07klx) on 8-h light, 16-h dark cycles for 2,4-DBE. Less light was used in the 2,4-DBE experiments to discourage excessive growth of filamentous green algae. Each CSTR was tested by introducing a continuous aqueous concentrate of the xenobiotic chemical until a steady-state concentration was observed for several days. Various organic and/or inorganic nutrients were continuously

added to separate CSTRs to achieve a large diversity of microbial concentrations and community structures (1,2). The channels were inoculated weekly using water samples from local lakes and streams. Between studies for each xenobiotic chemical, the channels were cleaned and washed with sodium hypochlorite solution.

Field Sites

The field sites consisted of both lotic and lentic freshwater systems and ranged from a mountain stream to a coastal bayou. The 11 sites were the Alabama River at highway 10 near Camden, AL; Harris Shoals Dam, a small pond in Oconee County, GA; High Shoals, a shallow shoals area in Oconee County, GA; Lassiter's Creek, a small creek in a wooded area in Oglethorpe County, Georgia; Oconee River in Whitehall, GA; a municipal sewage treatment plant in Athens, GA; Shope Fork Creek, a mountain stream in Coweeta, NC; the South Fork Broad River at Watson Mill State Park near Comer, GA; the St. Mary's River at the outflow of the Okefenokee Swamp at Sanderson, FL; Tom King Bayou near Gulf Breeze, FL; and Upper Three Runs Creek at the Savannah River Plant near Aiken, SC. The sites were sampled during May through August 1983.

RESULTS

Tables 1 and 2 summarize the transformation rate coefficient data for our laboratory microcosm and field-collected microbiota. Each of the test xenobiotic chemicals was rapidly transformed in all laboratory microcosm studies. 2,4-DBE was rapidly transformed in all field-collected microbiota samples; MP was transformed in 6 out of 10 sites; and DEP was transformed in only 2 out of 10 sites. Field-collected samples that did transform DEP and MP, however, did so at similar rates per unit of periphyton-colonized area and had similarly wide k_b ranges compared to laboratory microcosm data.

Blended aufwuchs that did not transform DEP or MP were observed for

24 h. No detectable transformation occurred even though very high concentrations of microbiota were used such that transformation half-lives were expected to be less than 1 h based on other studies with microbial samples that did transform the chemicals. Similarly, attached periphyton that did not transform DEP or MP were observed for 5 days. Aufwuchs samples taken from South Fork Broad River and High Shoals in 1980 rapidly transformed MP; however, samples collected from these sites for this present study three years later did not transform MP. When blended aufwuchs samples from the two sites were centrifuged and washed several times with distilled water then suspended in sterile river water before being spiked with MP, the microbiota rapidly transformed MP. When the samples were suspended in blended aufwuchs supernatant, rather than river water, the microbiota did not transform MP.

Washing blended aufwuchs samples and suspending them in river water did not result in transformation of DEP. One aufwuchs sample from South Fork Broad River was blended and 30 bacterial isolates from the sample were tested for their capacity to transform DEP in pure cultures according to a previously published procedure (1). None of the isolates transformed DEP, whereas, usually about 50% of bacterial isolates rapidly transformed DEP in the earlier study (1). A Brevibacterium isolate that transformed DEP in our earlier microcosm studies still rapidly transformed DEP after 3 years of weekly transfers on nutrient agar slants. Supernatant of blended aufwuchs samples from South Fork Broad River did not affect DEP transformation for 24 h by the Brevibacterium isolate.

DISCUSSION

The microbial transformation of our test xenobiotic chemicals appeared to be an "all or none" phenomenon. Transformation either proceeded at

similar rates per unit of periphyton-colonized area among diverse field and laboratory microcosm environments, or it did not proceed at all. Likewise, blended aufwuchs that transformed the chemicals had similar k_b ranges, i.e. similar transformer to non-transformer community structures among field and laboratory microcosm samples, or had no detectable transformer bacteria present, as was the case with the South Fork Broad River sample tested for DEP transformer bacteria. Continuing to follow the "all or none" observation, MP transformation proceeded rapidly in blended aufwuchs samples from South Fork Broad River and High Shoals washed and suspended in river water, but no detectable transformation occurred in the presence of the blended aufwuchs supernatant.

The absence of MP transformation in some field-collected samples was obviously not a lack of adaptation to MP, but was suppression of transformation by an agent that could be removed by centrifuging blended aufwuchs samples and washing them with distilled water. Aufwuchs could possibly cause a diauxic effect by releasing a nutrient source taken up preferentially to MP; however, diauxie did not occur upon the addition of glycerol, low concentrations of glucose, or nutrient broth (1). Aufwuchs samples were dominated by algae; however, no correlation between suppression of MP transformation and dominant algae in aufwuchs was evident. Suppression occurred in field samples dominated by a green alga, Spirogyra sp. (South Fork Broad River) and in samples dominated by diatoms, including Tabellaria, Gomphonema, and Navicula (Upper Three Runs Creek) and Frustulia and Emotia (St. Mary's River).

Transformation was selectively suppressed: MP transformation was suppressed whereas 2,4-DBE transformation proceeded rapidly at expected rates. This selectivity suggests that the suppression resulted from repression of the gene responsible for synthesizing the enzyme for MP transformation, or by

inhibition of the enzyme. It has become common knowledge that microbial transformations of xenobiotic chemicals in sediment microcosms may not occur for several days or weeks and then suddenly proceed at very rapid rates. Although no studies have been published elucidating this phenomenon, the delay is usually attributed to microbial adaptation to the xenobiotic chemical. Adaptation could occur at the cellular level by induction of enzymes or at the community level by development of a significant transformer population via mutation and/or selection. None of these processes, however, appear to explain the sudden rapid transformation after days or weeks of inactivity.

Enzyme induction by the presence of the xenobiotic chemical, if it occurs similarly to classical induction, should occur within minutes after adding the inducer (4). Also, sudden rapid transformation does not suggest population selections that are constrained by the growth rates of the organisms. Because sediment samples, like aufwuchs, include high concentrations of diverse microbiota, the phenomenon may be explained as repression of the genes or inhibition of the enzymes by metabolic products of microorganisms.

Suppression of transformation can occur not only from microbial sources, but also as a result of other xenobiotic chemicals present in the samples in very low concentrations. As little as 20 μg $liter^{-1}$ DEP stopped MP transformation in our aufwuchs samples and in pure cultures of bacteria transforming MP. Johnson (5) has reported similar findings with mixtures of xenobiotic chemicals.

We were not able to demonstrate suppression of DEP transformation by agents present in aufwuchs samples. On the contrary, the problem appeared to be an absence of DEP transforming bacteria. We have shown that DEP transforming bacteria can be out-competed when easily utilizable substrates are added to aufwuchs samples (1, 2). Perhaps this process could occur over

sufficient time to virtually extinguish particular xenobiotic transformers from an environment. Perhaps also, industrial or natural biological toxins could even destroy particular transformer populations or permanently incapacitate organisms from carrying out selected transformations.

Suppression of transformation, whether resulting from the presence of inhibitors, community changes, diauxie, or from unknown processes, is a surprisingly common observation in aufwuchs based on our experience using field-collected samples. Like adaptation, it is a phenomenon that diminishes our ability to predict microbial transformation rates of environmental pollutants with certainty. While adaptation is expected to be more environmentally significant as the concentration of a particular pollutant increases, suppression of transformation would be more important as the concentrations of yet unidentified microbial species and additional pollutants that cause the phenomenon increase. Therefore, suppression of transformation is probably much less predictable than adaptation for soil/sediment and aufwuchs-dominated systems.

Although suppression of transformation appears to be a serious problem relating to transformation of xenobiotic chemicals within our 5-day test period, additional studies need to be done over longer spans of time to determine whether transformer populations or other conditions conducive to transformation (for example, degradation of inhibitors) would eventually develop. Also, suppression of transformation may not be a significant concern in open waters where the concentrations of microbiota and pollutants are sufficiently dilute. This hypothesis is supported by the rapid transformation of several xenobiotic chemicals in numerous field-collected water samples studied by Paris et al. (6).

CONCLUSIONS

Based on our observations using blended aufwuchs and intact periphyton in laboratory microcosm and field-collected samples, we have reached the following conclusions concerning the use of laboratory microcosms for predicting microbial transformation rates in environmental situations.

(i) Transformation rate coefficients for hydrophilic, relatively easily transformable chemicals such as 2,4-DBE, DEP and MP, are similar for microbiota in laboratory microcosms and natural environmental waters.

(ii) Microbiota that transform such chemicals in microcosms can have similar complexities in community structures or transformer to nontransformer organisms as microbial communities in field situations.

(iii) Because transformations can be suppressed in the presence of high concentrations of microbiota, researchers using microcosms containing high microbial concentrations in soil, sediment, or aufwuchs cannot predict whether certain xenobiotic chemicals (such as MP and DEP) will be immediately transformed in a particular field site that is dominated by high concentrations of diverse microbiota.

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Table 1. Transformation rate coefficients for blended aufwuchs (k_b) and attached periphyton (k_A) in laboratory microcosms[a].

Microcosm	Methyl Parathion				Diethyl Phthalate				2,4-D Butoxyethyl Ester			
	n^b	k_b^c	n^d	k_A^e	n^b	k_b^c	n^d	k_A^e	n^b	k_b^c	n^d	k_A^e
A1									2	0.86±0.16	3	10.0±0.80
A2									1	1.5	3	9.7±0.46
A3	2	0.0033±0.00004	3	0.60±0.16	3	0.68±0.16	3	3.6±0.81	2	0.76±0.04	3	23.0±3.4
A4	1	0.0022	3	0.93±0.49	3	0.35±0.10	3	4.4±0.12	2	0.36±0.08	3	22.0±1.4
A5	2	0.0012±0.00014	3	1.3±0.91	3	0.03±0.01	3	10.0±.17				
A6	1	0.012	3	2.2±0.70	3	0.11±0.01	3	6.0±0.55				
A7	1	0.0091	3	1.9±0.51	3	0.15±0.05	3	4.6±0.50				
A8	1	0.010	3	2.6±1.0	3	0.18±0.03	3	5.4±0.60				
B1									1	0.64	3	16.0±2.6
B2											3	14.0±0.52
B3	1	0.0026	3	1.1±0.35	1	0.77	3	4.0±0.40	2	13.0±1.4	3	20.0±3.4
B4	1	0.0019	3	0.92±0.34	3	2.2±0.14	3	4.4±0.12	2	0.17±0.03	3	21.0±2.9
B5	2	0.0064±0.00014	3	1.6±0.12	3	0.01±0.001	3	10.0±0.36				
B6	1	0.0011	3	1.2±0.55	3	0.04±0.01	3	5.0±0.21				
B7	1	0.0011	3	1.5±0.72	3	0.23±0.05	3	3.0±0.15				
B8	2	0.0083±0.0025	3	1.6±0.12	3	0.20±0.01	3	1.7±0.00				
k_A Mean ± S.D.:				1.5±0.57				5.2±2.5				17.0±5.3

[a] microcosms were cleaned out and disinfected between studies with each chemical

[b] number of batch cultures

[c] $nl\ cell^{-1}\ h^{-1}$ based on total plate counts

[d] number of steady-state xenobiotic chemical concentration determinations

[e] liters $m^{-2}\ h^{-1}$; water velocity: 0.0425 m sec^{-1} near periphyton surface

Table 2. Transformation rate coefficients for blended aufwuchs (k_B) and attached periphyton (k_A) for field-collected microbiota[a].

Site	Methyl Parathion				Diethyl Phthalate				2,4-D Butoxyethyl Ester			
	$\underline{n}^b$	k_b^c	$\underline{n}^d$	k_A^e	$\underline{n}^b$	k_b^c	$\underline{n}^d$	k_A^e	$\underline{n}^b$	k_b^c	$\underline{n}^d$	k_A^e
Alabama River	3	0.0015±0.0003			1	0.004			2	0.18±0.06		
Harris Shoals Dam	3	0.00			3	0.00			5	5.6±2.2		
High Shoals	10[f]	0.08±0.07			3	0.00						
Lassiter's Creek	3	0.00			3	0.00			3	3.8±1.2		
Oconee River	3	0.00	2	0.80±0.00	3	0.00	3	0.00			3	32.0±6.4
Sewage Treatement Plant	3	0.0014±0.0004	2	1.8±0.16			2	3.8±2.1			3	60.0±3.5
Shope Fork Creek											3	14.0±4.0
South Fork Broad River	9[f]	0.18±0.19	2	0.77±0.05	3	0.00			7	6.2±5.4	2	20.0±2.8
St Mary's River	3	0.00	2	0.00	3	0.00			2	1.2±0.66	3	16.0±3.2
Tom King Bayou	3	0.25±0.08	2	0.45±0.10	3	0.00	2	0.00	3	40.0±9.5	3	18.0±0.0
Upper Three Runs Creek	2	0.11±0.01	1	0.80	2	0.00	2	0.00	2	9.4±3.7	3	31.0±5.2
		k_A mean ± S.D.:		0.92±0.51				3.8				27±16

a Teflon strips were colonized for 10 weeks

b number of batch cultures

c nl $cell^{-1}$ h^{-1} based on total plate counts

d number of steady-state xenobiotic chemical concentration determinations.

e liters m^{-2} h^{-1}; water velocity: 0.0425 near periphyton surface

f samples tested in 1980

Figure 1

Calculated data for substrate concentrations, [S], versus time, t, using the Michaelis-Menten equation for a V_{MAX} of 0.008 mg/liter^{-1}h^{-1} and K_m equal to 0.08 mg/liter.

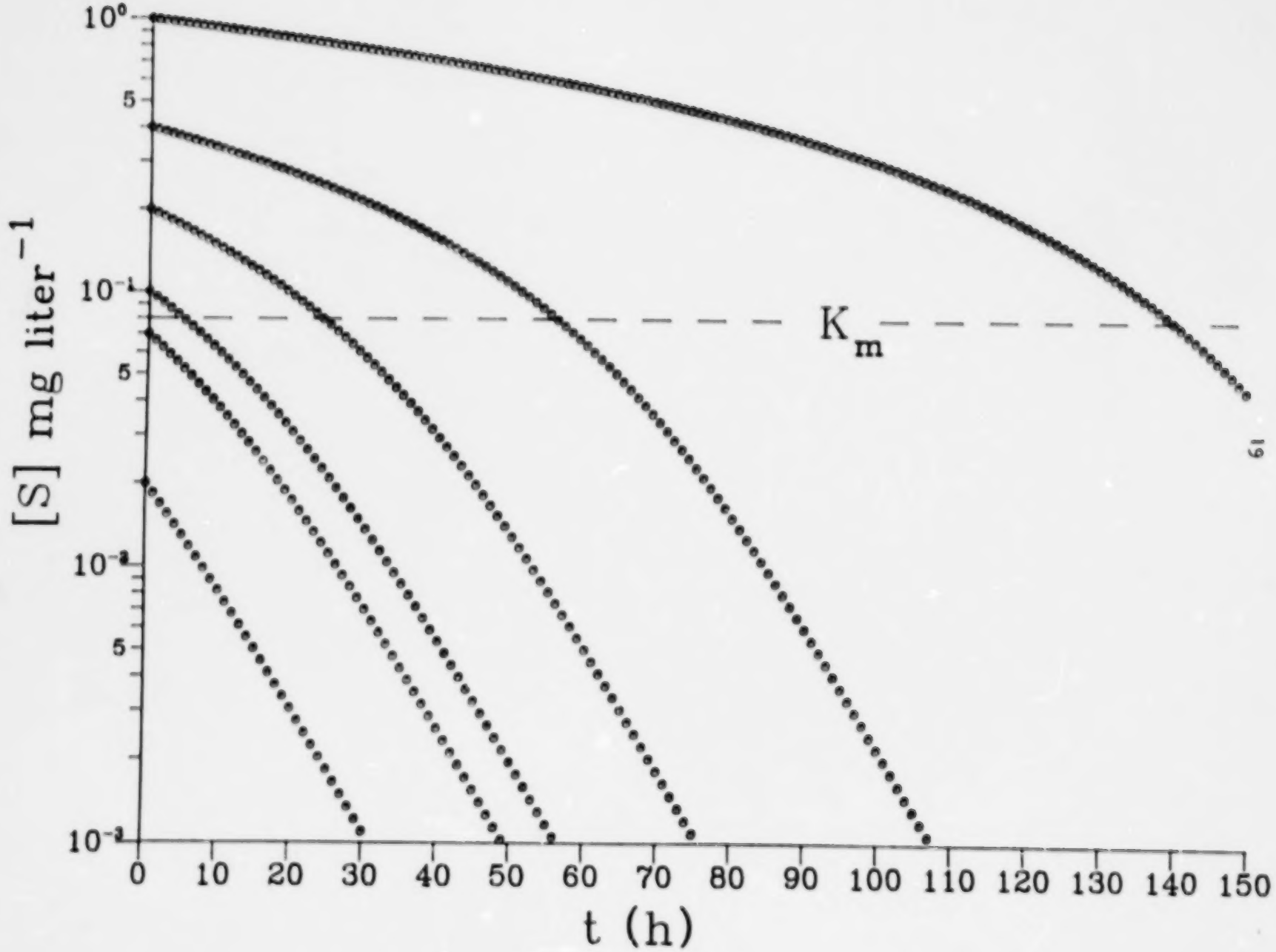

10^0
5
10^{-1}
5
10^{-2}
5
10^{-3}
K_m
[S] mg liter^{-1}
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150
t (h)

Figure 2

Loss of 2,4-DBE versus time for periphyton-colonized Teflon strip. Water velocity: 0.0425 m sec^{-1}.

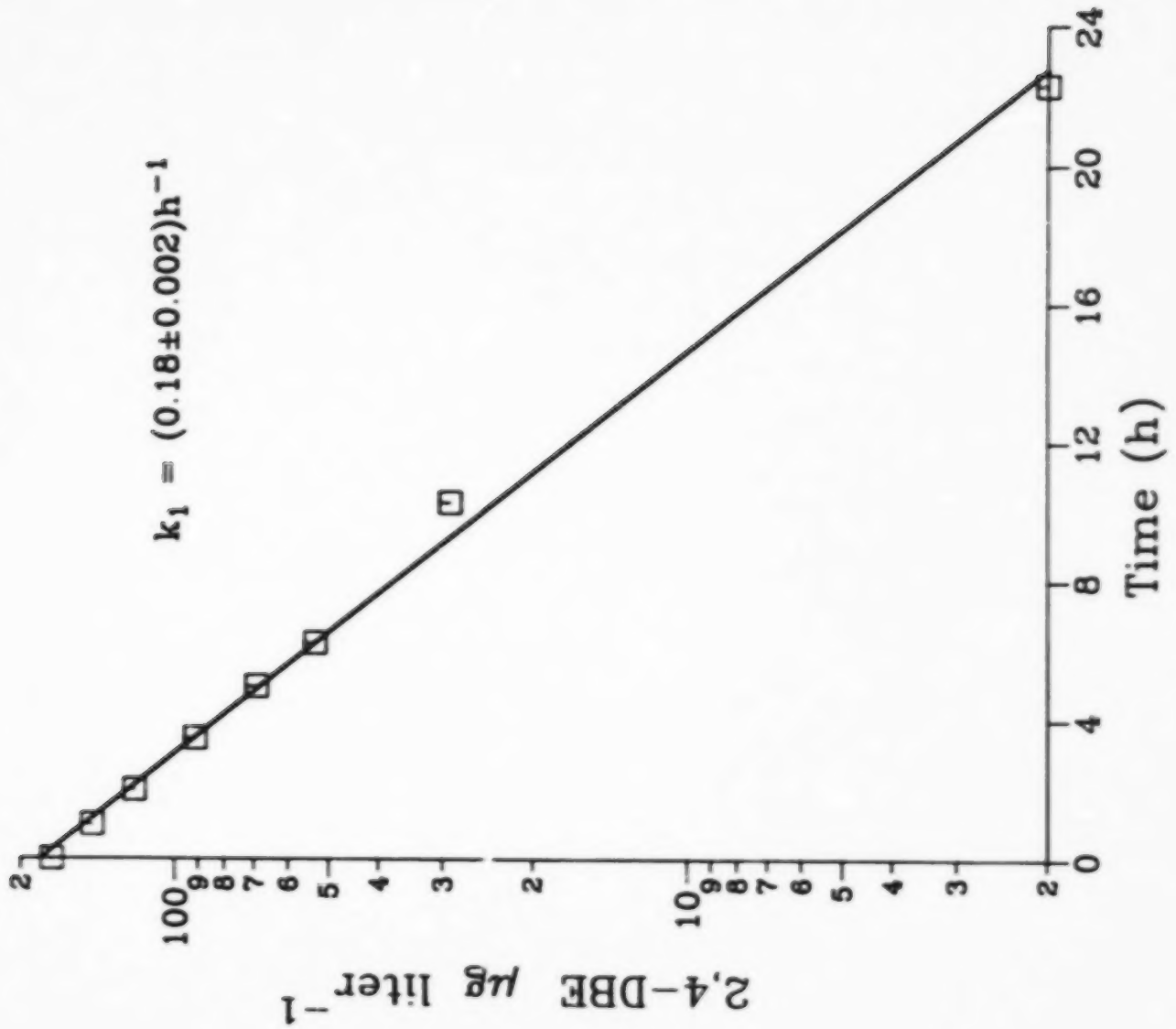

$k_1 = (0.18 \pm 0.002) h^{-1}$
2,4-DBE μg liter^{-1}
Time (h)
0
4
8
12
16
20
24
100
10
2
3
4
5
6
7
8
9

Figure 3

First-order transformation rate coefficients, k_1, of 2,4-DBE were directly proportional to turbulence measured in Reynolds numbers over the range tested. Water velocity: 0.0212 to 0.0755 m sec $^{-1}$. Teflon strips were colonized in a laboratory microcosm for either 2 or 7 weeks to vary the thickness of periphyton (AFDW was 9 and 71 g m^{-2} respectively).

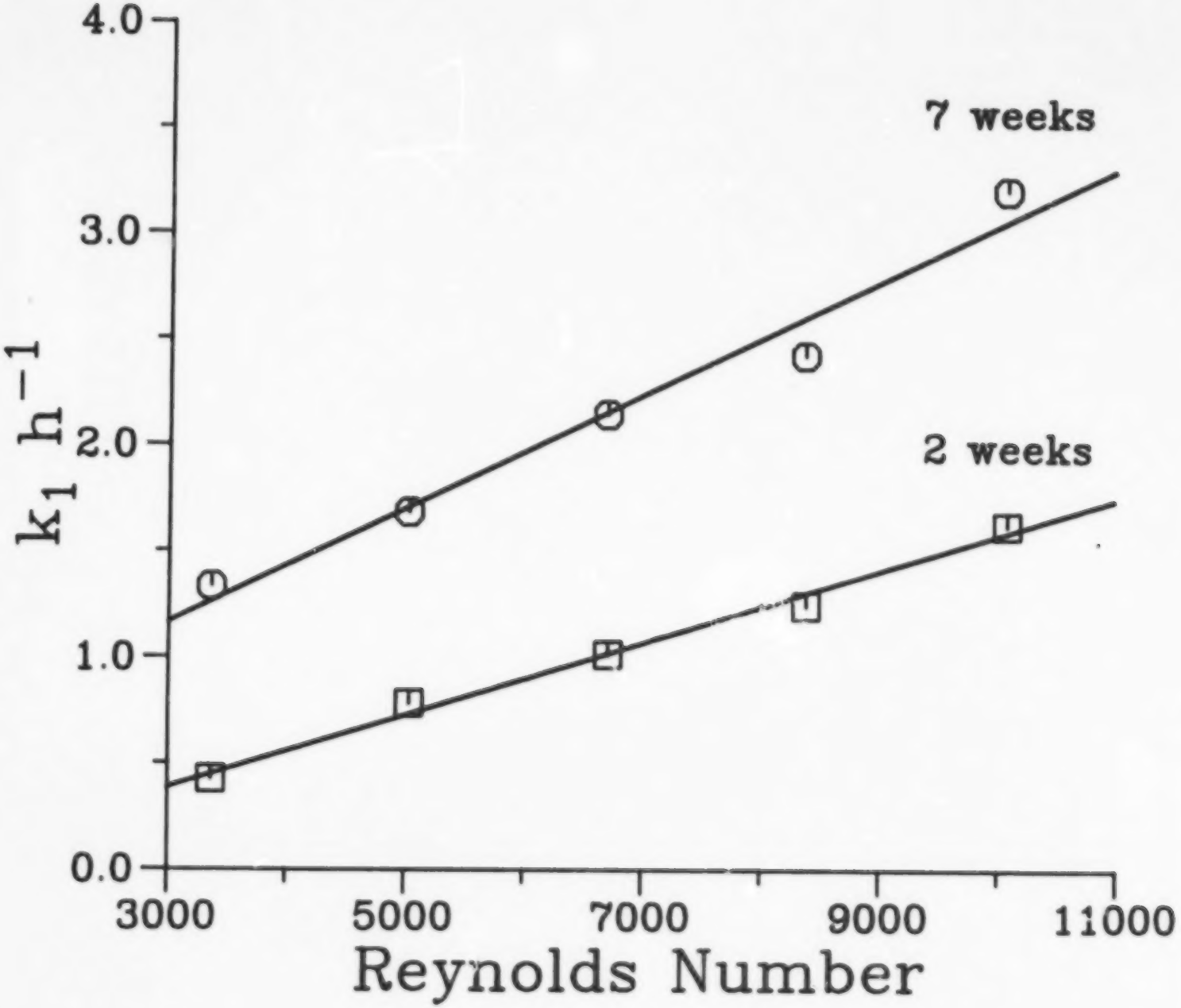
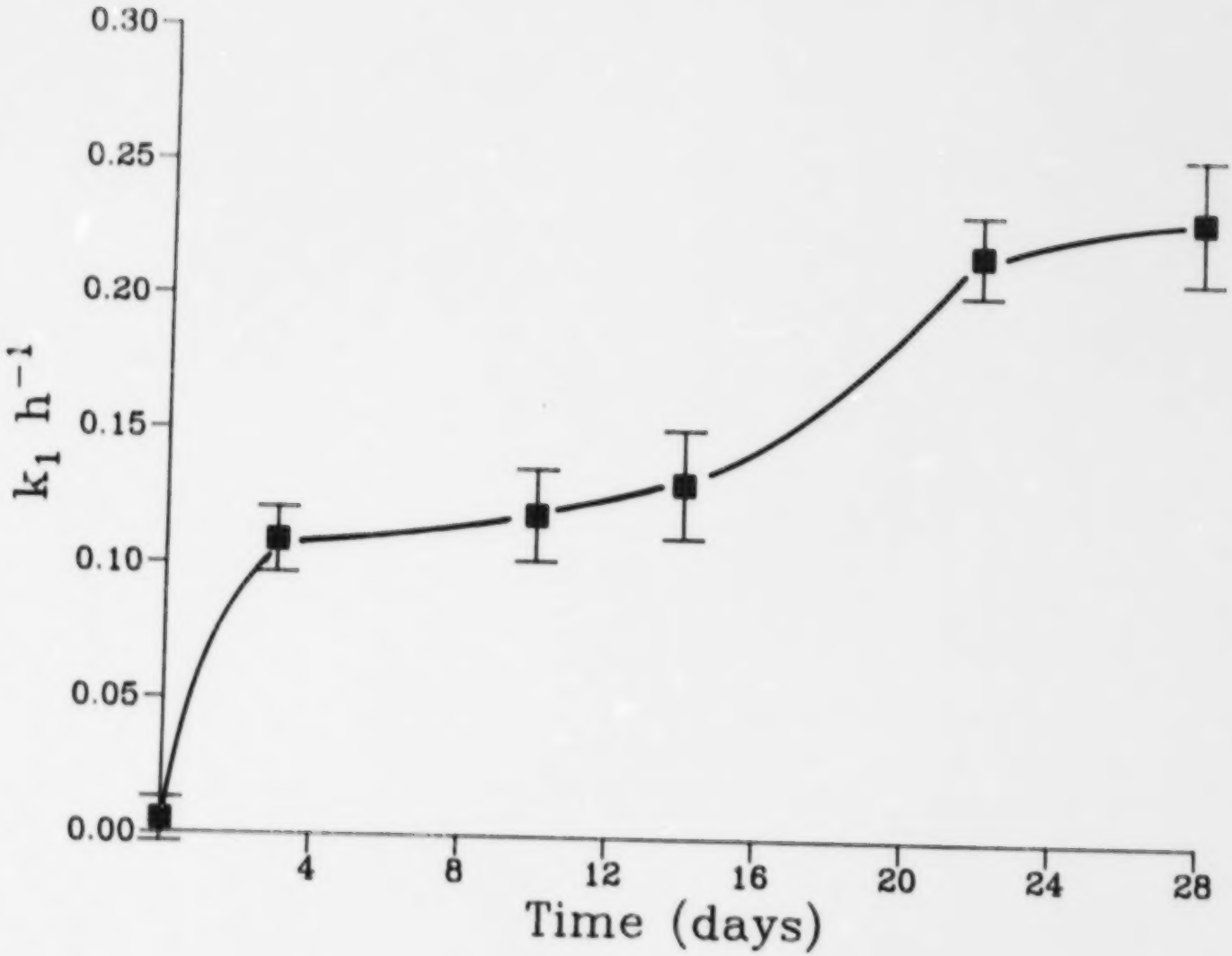

4.0
3.0
2.0
1.0
0.0
k_1 h^{-1}
7 weeks
2 weeks
3000
5000
7000
9000
11000
Reynolds Number

Figure 4

Colonization time versus first-order 2,4-DBE transformation rates. Teflon strips having equal surface areas were incubated for various lengths of time in a laboratory stream and their rate coefficients of 2,4-DBE loss, k_1, determined. Water velocity: 0.0425 m sec^{-1}


0.30
0.25
0.20
0.15
0.10
0.05
0.00
k_1 h^{-1}
4
8
12
16
20
24
28
Time (days)